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(54) TASTE-RELATED PROTEIN EXTRACTANT

PURPOSE: To obtain a taste-related protein extractant capable of extracting a taste-related protein which exists on the tongue and plans an important role in manifestation of sweetness, flavor, etc., and of changing taste sensitivity of the tongue.

CONSTITUTION: This taste-related protein extractant comprises a suspension of liposome containing an amide bond-containing phosphatidylcholine of formula I (R1 and R2 are 8-24C alkyl or 8-24C alkenyl), preferably 1,2-

dimyristoylamido-1,2-deoxyphosphatidylcholine(DDPC). The suspension of liposome comprises the compound of formula I and a phospholipid for forming an ordinary liposome, preferably a phosphatidylcholine of formula II (R3 and R4 are R1 and R2) such as egg yolk lecithin or dimyristoylphosphatidylcholine(DMPC) and a mixture of DDPC and egg yolk lecithin or DMPC in the molar ratio of 4:6 may be cited as the suspension of liposome. The extractant is brought into contact with the surface of epithelium of the tongue to extract a taste-related protein existing on the surface of epithelium of the tongue.

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CLAIMS

[Claim(s)]

[Claim 1] The following general formula (1)

It is the gustation intervention protein extractant which consists of the liposome suspension containing the phosphatidylcholine which has amide combination expressed with (R1 and R2 show the alkyl or alkenyl machine of carbon numbers 8-24 among a formula).

[Claim 2] Furthermore, liposome suspension is the following general formula (2).

It is the gustation intervention protein extractant according to claim 1 which is a thing containing the phosphatidylcholine expressed with (R3 and R4 show the alkyl or alkenyl machine of carbon numbers 8–24 among a formula).

[Claim 3] How to extract the gustation intervention protein which carries out actual [of contacting a gustation intervention protein extractant according to claim 1 or 2 on a tongue epithelium front face] to the tongue epithelium front face by which it is characterized.

[Claim 4] The measuring method of gustation sensitivity change characterized by measuring potential change of a glossopharyngeal nerve before and after contacting a gustation intervention protein extractant according to claim 1 or 2 on a tongue epithelium front face.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] Actual [of this invention] can be carried out on a tongue, it can extract the gustation intervention protein which is playing the role important for a manifestation of sweet taste, ******, etc., and relates to the extraction technique using the gustation intervention protein extractant and this to which the gustation sensitivity of a tongue can be made to change.

[0002]

[Description of the Prior Art] As matter which stimulates the taste cells on a tongue, an acid, a salt, a bitter substance, the sweet taste matter, the ****** matter (amino acid), etc. are known. Among these, a salty taste, acidity, and bitterness are called taste which these causative agents acted and discover to a tongue top lipid membrane. For example, about the relation between a bitter substance and a tongue top lipid membrane, since the response threshold to the various bitter substances of asolectin liposome and human being's gustatory threshold show good functionality, it turns out that the acceptance site of the bitter substance in taste cells is the same hydrophobic site as a lipid dyad layer [a layer (MEMBRANE) and 13(3).144-151 (1988)].

[0003] Based on this knowledge, about the bitter substance, the acid, and the salt, a common lipid dyad layer is used and the research is advanced.

[0004] On the other hand, if a tongue is processed by the proteolytic enzyme, since a nervus response will disappear about sweet taste and a taste, a specific receptor protein minds [the sweet taste matter and / taste], and it is thought that the response is discovered.

[0005] However, this tongue top protein exists in a tongue front face, since it is a membrane protein further, the extraction is difficult, and the research is seldom progressing.
[0006]

[Problem(s) to be Solved by the Invention] Therefore, it was difficult to have made the gustation sensitivity of the tongue about sweet taste and the taste matter change except for the case where a proteolytic enzyme is used. Therefore, the purpose of this invention tends to offer the technique of extracting gustation intervention protein, tends to give change to the gustation sensitivity of a tongue, and tends to use for uptake adjustment of the sweet taste matter etc.
[0007]

[Means for Solving the Problem] When contacting on the tongue the liposome suspension which contains the phosphatidylcholine expressed with the following general formula (1) as a result of this invention persons' inquiring zealously in view of ** or *****, gustation intervention protein could be extracted, after the extraction, it found out that the gustation about sweet taste and a taste decreased, and this invention was completed.

[0008] That is, this invention is the following general formula (1).

[Formula 3]
$$0$$
 CH_3 $\frac{1}{1+}$ CH_3 $\frac{1}{1+}$ CH_3 $\frac{1}{1+}$ CH_3 $\frac{1}{1+}$ CH_3 $\frac{1}{1+}$ CH_4 CH_5 CH_5 CH_5 CH_5 CH_5 CH_5 CH_5

[0010] The gustation intervention protein extractant which consists of the liposome suspension containing the phosphatidylcholine which has amide combination expressed with (R1 and R2 show the alkyl or alkenyl machine of carbon numbers 8–24 among a formula) is offered. Moreover, the technique of extracting the gustation intervention protein which carries out actual [of this invention contacting the concerned gustation intervention protein extractant on a tongue epithelium front face] to the tongue epithelium front face by which it is characterized is offered. Furthermore, the measuring method of gustation sensitivity change characterized by this invention measuring potential change of a glossopharyngeal nerve before and after contacting the concerned gustation intervention protein extractant on a tongue epithelium front face is offered again.

[0011] R1 of the phosphatidylcholine which has amide combination expressed with the general formula (1) used by this invention And R2 Although the carbon numbers of the alkyl or alkenyl machine shown are 8-24, especially the thing of 10-20 is desirable. Specifically, a lauroyl machine, a myristoyl machine, a PAL ***** machine, etc. are desirable. As phosphatidylcholine which has a desirable amide combination especially, DDPC

(1,2-dimyristoylamide-1,2-deoxyphosphatidyl choline) is mentioned. Moreover, the phosphatidylcholine (1) which has

amide combination can be manufactured by technique given in JP,61-267509,A. [0012] This phosphatidylcholine (1) and technique better known than the usual phospholipid for liposome formation, for example, a representation target, can manufacture the liposome suspension in this invention according to a vortex process (Vortexing method or Hydration method) [Bangham, A.D., Standish, H.H.& Hatkins, J.C.J.Hol.Biol., 13,238 (1965)]. Although lipids, such as lecithin more specifically extracted and refined from a soybean, the yolk, etc. as the above-mentioned phospholipid for liposome formation and a sphingomyelin, and the synthetic lipid which has dipeptide combination near the polar head are mentioned, it is the following general formula (2). [0013]

[0014] The phosphatidylcholine expressed with (R3 and R4 show the alkyl or alkenyl machine of carbon numbers 8-24 among a formula), for example, yolk lecithin, dimyristoyl phosphatidyl choline (DMPC), etc. are desirable. [0015] As desirable combination of the phosphatidylcholine (1) which has amide combination, and the usual phospholipid for liposome formation, what set DDPC, yolk lecithin, or DDPC and DMPC to about 4:6 (mole ratio) is mentioned. The liposome suspension carried out and obtained can be used as a gustation intervention protein extractant like the above.

[0016] This extraction can be carried out by contacting the above-mentioned liposome suspension on a tongue epithelium front face. What is necessary is just to specifically repeat and pour out liposome suspension on a tongue. Although the tongue applied here is organum gustus to which protein participates in the tongue of an animal, and a gustation manifestation, in especially this invention, its tongue of a mammal, an amphibian, a reptile, birds, and fishes is desirable. Moreover, the amount of the liposome suspension used to a tongue is very important about the amount of extraction protein, for example, it is desirable to add 10ml of the liquid with which DDPC:DMPC=4:6 [5mg] are contained in 1ml of liposome suspension to the tongue of one ****, and it is desirable to add 30ml of the liquid with which DDPC:DMPC=4:6 [15-45mg] are contained in the Homo sapiens.

[0017] The gustation intervention protein carried out and extracted like the above is reconstructed on the liposome, with activity held, and this can be used as the gustation and a gustation intervention protein research, for example, an artificial-membrane taste sensor etc.

[0018] Moreover, before and after contacting the liposome suspension and the tongue epithelium front face of this invention, change of a gustation sensitivity can be measured by measuring potential change of a glossopharyngeal nerve. Since a salt and bitterness come to be sensed strong by using the liposome suspension of this invention before a meal as a collutory, it stops sensing a saccharinity further by the ability reducing the salt in food and sweet taste food becomes unsavory, the application side that the ingestion of sweet taste food can be checked is also considered.

[Effect of the Invention] The extract which the gustation intervention protein extractant of this invention can extract easily the gustation intervention protein which participates in a manifestation of the sweet taste on a tongue, ******, etc., and was obtained can be used for a research of gustation intervention protein. Furthermore, change of a gustation sensitivity can be known by measuring potential change of a glossopharyngeal nerve, before and after contacting the gustation intervention protein extractant of this invention on a tongue epithelium front face. On the other hand, by blending with the mouthwash etc., the gustation intervention protein extractant of this invention can change the gustation sensitivity of a tongue, and can also expect effects, such as sweet taste matter ingestion suppression.

[Example] Although an example is given and this invention is explained still in detail hereafter, this invention is not

[0021] According to technique (W.S.Singleton, H.S.Gray, H.L.Brown, J.L.White, J.Am.Oll Chem.Soc., and [42, 53] (1965)) given in reference, the yolk phosphatidylcholine (EggPC) was extracted from the example 1 yolk, and it refined using the alumina column. The purity was checked by TLC. Moreover, DDPC was compounded from 2 and 3-diamino propionic acid by technique given in JP,61-267509,A [Junzo Sunamoto et al., the Chemical Society of Japan, 1987 (3), p 569-574]. 10.6mg of the above-mentioned EggPC and 7.0mg of DDPC were mixed, and it was made to melt into 2ml chloroform in an eggplant type flask. It distilled off under reduced pressure of chloroform using the rotating evaporator, and overnight neglect was further carried out in the vacuum desiccator. Subsequently, on the vortex mixer, 1ml of PBS (pH7.38) was made to swell the obtained liquid, and it was stirred. Then, using the UR200P probe type ultrasonic-wave crusher (made in Tommy), the ultrasonic irradiation was carried out to the bottom of a nitrogen gas draft by 0 degree C and 25W for 5 minutes, and desired liposome suspension was obtained. [0022] Example 2 It anesthetizes at extraction:**** of ****** top protein (urethane anesthetization), and a tongue is pulled out from the inside of an oral cavity, and a gustation sensing side is turned up and it fixes. Then, the liposome suspension to which 1 hour repeat deed tongue top gustation intervention protein reconstructed the operation which adds with a dropper 10ml of the liposome aqueous solutions which contain 5mg of the lipids of DDPC:DMPC=4:6 in 1ml on a tongue, receives this solution with a laboratory dish on the reverse side of a tongue, inhales this solution with a dropper, and is added on a tongue was obtained.

[0023] Example 3 The silver—silver chloride electrode was connected with the glossopharyngeal nerve of effect:**** of the liposome for a tongue top protein extraction, gustation response change was measured as nervus potential change, and measured value was compared before and after performing liposome processing shown in the example 2 for 1 hour. The result is shown in <u>drawing 1 - view 4</u>. About the shoe cloth and 1-alanine which are a sweet taste component, the clear decrement was seen immediately after processing from the <u>drawing 1</u> and the <u>drawing 2</u> compared with nervus potential variation's at the time of carrying out gustation stimulus processing before. Then, as a result of separating the liposome in the obtained liposome suspension and carrying out determination of the amount of extraction proteins in a liposome using a fluorescence indicator (full run), it checked that 133microg [/ml] protein was extracted.

[0024] Example 4 The after [analysis] comparison study of the protein which could wash the tongue by ion exchange water before the liposome extract mentioned as the analysis:example 2 of extraction protein and the liposome extraction was carried out by SDS-PAGE. The result is shown in the <u>drawing 5</u> and the <u>drawing 6</u>. From drawing, a difference is clearly accepted in the amount (60 or more KDs) of macromolecules, and it became clear that protein is

extracted by liposome suspension.

[0025] example 5 alternative masking [of the gustation by optimization of an extraction condition]: — generally a salt, an acid, and bitterness are called taste acted and discovered to a tongue top lipid membrane, and it is thought that it is the taste which acts on the receptor protein considered that ****** and a taste exist on a tongue, and is discovered Then, the liposome extraction method which does not damage a tongue top lipid but mainly extracts the receptor protein on a tongue was examined using the liposome mentioned as the example 2. A result is shown in drawing 7. Consequently, when extracting by making extraction time into 30 minutes, it turns out that only sweet taste can be alternatively masked about immediately after an extraction.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] In an example 3, it is drawing in which being before and after processing of this invention extractant, and showing the variation of the nervus potential at the time of carrying out L-alanine stimulus.

[Drawing 2] In an example 3, it is drawing in which being before and after processing of this invention extractant, and showing the variation of the nervus potential at the time of carrying out a shoe cloth stimulus.

[Drawing 3] In an example 3, it is drawing in which being before and after processing of this invention extractant, and showing the variation of the nervus potential at the time of carrying out a quinine hydrochloride stimulus.

[Drawing 4] In an example 3, it is drawing in which being before and after processing of this invention extractant, and showing the variation of the nervus potential at the time of carrying out L-leucine stimulus.

[Drawing 5] In an example 4, it is drawing showing the analysis result of the protein which washed and obtained the tongue by ion exchange water.

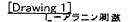
[Drawing 6] In an example 4, it is drawing showing the analysis result of the extracted protein by this invention extractant.

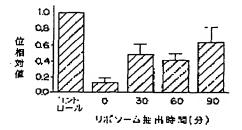
[Drawing 7] In an example 5, it is drawing showing the result of alternative masking of the gustation by this invention extractant.

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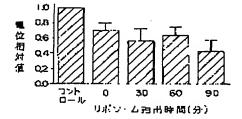
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DRAWINGS

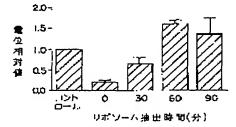




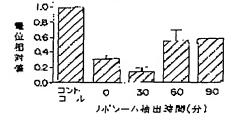
[Drawing 2] シェクコ ス刺激



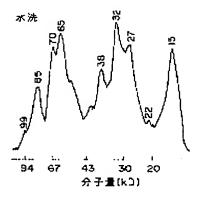
[Drawing 3] : 1 - 1 - 2 · 監神医判面

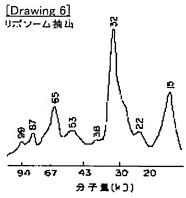


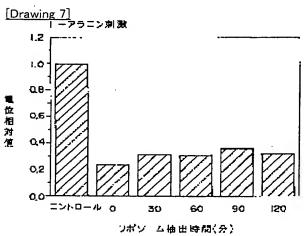
[Drawing 4]



[Drawing 5]







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(54) 【発明の名称】味覚関与蛋白質抽出剤

(57)【要約】

【構成】 下記一般式(1)

【化1】

(式中、R'及びR'は炭素数8~24のアルキル又はアルケニル基を示す)で表わされるアミド結合を有するホスファチジルコリンを含むリボソーム懸濁液からなる味覚関与蛋白質抽出剤。

【効果】 舌上の甘味、うまみ等の発現に関与する味覚 関与蛋白質を容易に抽出することができる。 【特許請求の範囲】

【請求項1】 下記一般式(1)

$$\begin{array}{c} \text{CH}_{2}-\text{O} & \text{CH}_{3} \\ \text{II} & \text{CH}_{2}-\text{O} & \text{CH}_{2} \\ \text{CH}_{2}-\text{O} & \text{CH}_{2} \\ \text{CH}_{3} & \text{CH}_{3} \\ \text{CH}-\text{NH} & \text{C}-\text{R}^{1} \\ \text{CH}_{2}-\text{NH} & \text{C}-\text{R}^{2} \\ \text{II} & \text{O} \\ \text{CH}_{2}-\text{NH} & \text{C}-\text{R}^{2} \\ \text{II} & \text{O} \\ \end{array}$$

(式中、R'及びR'は炭素数8~24のアルキル又はアルケニル基を示す)で表わされるアミド結合を有するホスファチジルコリンを含むリボソーム懸濁液からなる味覚関与蛋白質抽出剤。

【請求項2】 更に、リボソーム懸濁液が次の一般式(2)

$$\begin{array}{c} 0 & \text{CH}_{3} \\ | | \\ \text{CH}_{2} - 0 - \text{P} - 0 - \text{CH}_{2} \text{CH}_{2} - \text{N} - \text{CH}_{3} \\ | | | | \\ | | | | \\ \text{CH} - 0 - \text{C} - \text{R}^{3} \\ | | | | | | \\ \text{CH}_{2} - 0 - \text{C} - \text{R}^{4} \\ | | | | | \\ 0 \end{array}$$

(式中、 R^{3} 及び R^{4} は炭素数 $8\sim24$ のアルキル又は アルケニル基を示す) で表わされるホスファチジルコリンを含むものである請求項 1 記載の味覚関与蛋白質抽出 剤。

【請求項3】 請求項1又は2記載の味覚関与蛋白質抽出剤を舌上皮表面に接触させることを特徴とする舌上皮表面に顕在する味覚関与蛋白質を抽出する方法。

【請求項4】 請求項1又は2記載の味覚関与蛋白質抽 出剤を舌上皮表面に接触させる前後で舌咽神経の電位変 化を測定することを特徴とする味覚感受性変化の測定方 法。

【発明の詳細な説明】

[0001]

【産業上の利用分野】本発明は、舌上に顕在し、甘味、 うまみ等の発現に重要な役割を演じている味覚関与蛋白 質を抽出することができ、舌の味覚感受性を変化せしめ ることができる味覚関与蛋白質抽出剤及びこれを用いた 抽出方法に関する。

[0002]

【従来の技術】舌上の味細胞を刺激する物質としては、

酸、塩、苦味物質、甘味物質、うまみ物質(アミノ酸) 等が知られている。このうち、塩味、酸味、苦味はこれ らの原因物質が舌上脂質膜へ作用して発現する味と言わ れている。例えば、苦味物質と舌上脂質膜への関係につ いて、アゾレクチンリボソームの各種苦味物質に対する 応答閾値と人間の味覚閾値とが良い相関性を示すことか ら、味細胞における苦味物質の受容サイトは、脂質2分 子膜と同様な疎水性部位であることが判っている〔膜 (MEMBRANE),13(3).144-151.

10 (1988)).

【0003】この知見に基づき、苦味物質、酸、塩については、一般的な脂質2分子膜を利用し、研究が進められている。

【0004】一方、甘味、うま味については、舌を蛋白質分解酵素で処理すると神経応答が消失することから、甘味物質、うま味物質に特異的な受容蛋白質が介して応答が発現していると考えられている。

【0005】しかしながら、この舌上蛋白質は、舌表面に存在し、更に膜蛋白質であることから抽出が困難であ 20 り、研究はあまり進んでいない。

[0006]

【発明が解決しようとする課題】従って、蛋白質分解酵素を使用する場合を除いて、甘味、うま味物質についての舌の味覚感受性を変化せしめることは、困難であった。よって、本発明の目的は、味覚関与蛋白質を抽出する方法を提供し、舌の味覚感受性に変化を与え、甘味物質等の摂取調節等に役立てようとするものである。

[0007]

【課題を解決するための手段】斯かる実情に鑑み本発明 30 者らは鋭意研究を行なった結果、下記一般式(1)で表 わされるホスファチジルコリンを含むリボソーム懸濁液 を舌上に接触させれば、味覚関与蛋白質が抽出でき、抽 出後、甘味、うま味に関する味覚が減少することを見出 し本発明を完成した。

[0008] すなわち本発明は、下記一般式(1) [0009]

[化3]

【0010】(式中、R'及びR'は炭素数8~24のアルキル又はアルケニル基を示す)で表わされるアミド 50 結合を有するホスファチジルコリンを含むリボソーム懸

濁液からなる味覚関与蛋白質抽出剤を提供するものであ る。また、本発明は当該味覚関与蛋白質抽出剤を舌上皮 表面に接触させることを特徴とする舌上皮表面に顕在す る味覚関与蛋白質を抽出する方法を提供するものであ る。更にまた本発明は当該味覚関与蛋白質抽出剤を舌上 皮表面に接触させる前後で舌咽神経の電位変化を測定す ることを特徴とする味覚感受性変化の測定方法を提供す るものである。

【0011】本発明で用いる一般式(1)で表わされる アミド結合を有するホスファチジルコリンのR'及びR 10 'で示されるアルキル又はアルケニル基の炭素数は8~ 24であるが、特に10~20のものが好ましい。具体 的にはラウロイル基、ミリストイル基、パルミトイル基 等が好ましい。特に好ましいアミド結合を有するホスフ ァチジルコリンとしてはDDPC(1,2-ジミリスト イルアミドー1,2ーデオキシホスファチジルコリン) が挙げられる。また、アミド結合を有するホスファチジ ルコリン (1) は、特開昭61-267509号公報に 記載の方法により製造することができる。

【0012】本発明におけるリボソーム懸濁液は、この 20 ホスファチジルコリン (1) と通常のリボソーム形成用 リン脂質より、公知の方法、例えば代表的にはボルテッ クス法(Vortexing method又はHyd ration method) (Bangham, A. D., Standish, H. H. & Hatkin s, J. C. J. Hol. Biol., 13, 238 (1965)]に従って製造することができる。上記リ ボソーム形成用リン脂質としては、より具体的には例え ば、大豆、卵黄等から抽出、精製されたレシチン、スフ ィンゴミエリン等の脂質、極性頭部近くにジペプチド結 30 合を有する合成脂質が挙げられるが、次の一般式(2) [0013]

$$\begin{array}{c} \text{(AL 4)} \\ \text{CH}_2 - \text{O} & \text{P} - \text{O} - \text{CH}_2 \, \text{CH}_2 - \text{N} - \text{CH}_3 \\ | & \text{O} - & \text{CH}_3 \\ | & \text{O} - & \text{CH}_3 \\ \text{CH} - \text{O} - \text{C} - \text{R}^3 \\ | & \text{II} & \text{CH}_2 - \text{O} - \text{C} - \text{R}^4 \\ | & \text{O} \end{array}$$

【0014】 (式中、R3 及びR4 は炭素数8~24の アルキル又はアルケニル基を示す)で表わされるホスフ ァチジルコリン、例えば、卵黄レシチン、ジミリストイ ルホスファチジルコリン (DMPC) 等が好ましい。 【0015】アミド結合を有するホスファチジルコリン

(1)と通常のリポソーム形成用リン脂質の好ましい組

DMPCとを約4:6(モル比)としたものが挙げられ る。上記の如くして得られたリポソーム懸濁液は、味覚 関与蛋白質抽出剤として用いることができる。

【0016】この抽出は、上記リボソーム懸濁液を舌上 皮表面に接触させることにより実施することができる。 具体的にはリポソーム懸濁液を舌上に繰り返し注げばよ い。ここで適用される舌は、動物の舌及び味覚発現に蛋 白が関与する味覚器であるが、本発明では、哺乳類、両 生類、爬虫類、鳥類及び魚類の舌が特に好ましい。ま た、舌に対するリボソーム懸濁液の使用量は、抽出蛋白 質量に関して非常に重要であり例えば、牛蛙一頭の舌に 対してリポソーム懸濁液 1 ml中にDDPC: DMPC= 4:6が5mg含まれる液を10ml添加するのが好まし く、ヒトではDDPC:DMPC=4:6が15~45 mg含まれる液を30ml添加するのが好ましい。

【0017】上記の如くして抽出された味覚関与蛋白質 は、活性を保持したままリポソーム上に再構築されてお り、これは味覚及び味覚関与蛋白質研究、例えば人工膜 味センサー等として利用できる。

【0018】また、本発明のリボソーム懸濁液と舌上皮 表面を接触させる前後で、舌咽神経の電位変化を測るこ とにより味覚感受性の変化を測定することができる。本 発明のリボソーム懸濁液は、例えばうがい剤として食事 前に使用することにより、塩及び苦味が強く感じられる ようになるので、食品中の食塩を減らすことができ、更 に、甘さを感じなくなるため、甘味食品がまずくなるの で甘味食品の摂食を阻害することができるという応用面 も考えられる。

[0019]

【発明の効果】本発明の味覚関与蛋白質抽出剤は、舌上 の甘味、うまみ等の発現に関与する味覚関与蛋白質を容 易に抽出することができ、また、得られた抽出物は味覚 関与蛋白の研究に用いることができる。更に本発明の味 覚関与蛋白質抽出剤を舌上皮表面に接触させる前後で舌 咽神経の電位変化を測定することにより味覚感受性の変 化を知ることができる。一方、本発明の味覚関与蛋白質 抽出剤は、うがい薬等に配合することにより、舌の味覚 感受性を変化させることができ、甘味物質摂食抑制等の 効果も期待できる。

[0020]

【実施例】以下、実施例を挙げて本発明を更に詳細に説 明するが本発明は、これらに限定されるものではない。 【0021】実施例1

卵黄から文献記載の方法 [W. S. Singleto n, H. S. Gray, H. L. Brown, J. L. White, J. Am. Oll Chem. Soc., 42,53(1965)] に従い卵黄ホスファチジルコ リン(EggPC)を抽出し、アルミナカラムを用いて 精製した。その純度はTLCにより確認した。また、 合せとしては、DDPCと卵黄レシチン又はDDPCと 50 2,3-ジアミノブロピオン酸から特開昭61-267

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509号公報〔砂本順三ら、日本化学会誌、1987 (3)、p569-574] 記載の方法により、DDP Cを合成した。上記EggPCの10. 6mgとDDPC の7. 0mgとを混合し、ナス型フラスコ中で2mlのクロ ロホルム中に溶解させた。ロータリーエバボレーターを 用いてクロロホルムを減圧下に留去し、更に減圧デシケ ーター中で一夜放置した。次いで得られた液をボルテッ クスミキサー上でPBS (pH7.38) の1mlに膨潤さ せ、攪拌した。その後、UR200Pブローブ型超音波 間、0℃、25Wで超音波照射して、所望のリボソーム 懸濁液を得た。

【0022】実施例2 牛蛙舌上蛋白質の抽出:牛蛙 に麻酔(ウレタン麻酔)を施し舌を口腔内より引き出し 味覚感知面を上にして固定する。続いて1ml中にDDP C:DMPC=4:6の脂質5mgを含むリポソーム水溶 液10回をスポイドにより舌上に添加し、舌の裏でこの 溶液をシャーレにより受けこの溶液をスポイドで吸い舌 上に添加する操作を1時間繰り返し行ない舌上味覚関与 蛋白質の再構築したリボソーム懸濁液を得た。

【0023】実施例3 舌上蛋白質抽出用リポソーム の効果: 牛蛙の舌咽神経に銀・塩化銀電極を繋ぎ味覚応 答変化を神経電位変化として測定し、実施例2に示した リポソーム処理を1時間施した前後で測定値を比較し た。その結果を図1~図4に示す。図1及び図2より甘 味成分であるシュークロース及び1-アラニンについて は、味覚刺激した場合の神経電位変化量が処理前に比べ 処理直後では明らかな減少がみられた。続いて得られた リボソーム懸濁液中のリボソームを分離し、リボソーム 中の抽出蛋白量を蛍光指示薬(フルラン)を用いて定量 30 した結果、133μg/mlの蛋白質が抽出されているこ とを確認した。

【0024】実施例4 抽出蛋白質の分析:実施例2 に挙げたリポソーム抽出液とリポソーム抽出前に舌をイ

オン交換水で洗浄し得た蛋白質とをSDS-PAGEに より分析後比較検討した。その結果を図5及び図6に示 す。図より、明らかに高分子量(60KD以上)に違い が認められ、リボソーム懸濁液により蛋白質が抽出され ていることが判明した。

【0025】実施例5 抽出条件の最適化による味覚 の選択的マスキング:一般に、塩・酸・苦味は舌上脂質 膜へ作用して発現する味といわれ、他方甘・うま味は舌 上に存在すると考えられる受容蛋白質に作用して発現す 破砕機(トミー社製)を用いて、窒素ガス気流下に5分 10 る味であると考えられている。そこで、舌上脂質を傷つ けず舌上の受容蛋白質を主に抽出するリボソーム抽出法 について実施例2に挙げたリボソームを用い検討を行な った。結果を図7に示す。その結果、抽出時間を30分 として抽出を行なえば抽出直後について甘味だけを選択 的にマスキングできることが分かった。

【図面の簡単な説明】

【図1】実施例3において、本発明抽出剤の処理前後で L-アラニン刺激した場合の神経電位の変化量を示す図 である。

【図2】実施例3において、本発明抽出剤の処理前後で シュークロース刺激した場合の神経電位の変化量を示す 図である。

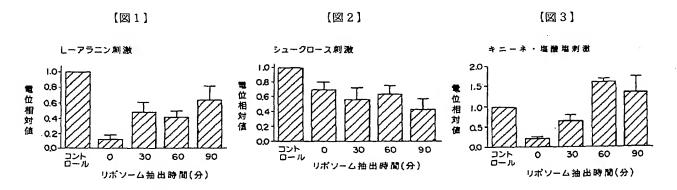
【図3】実施例3において、本発明抽出剤の処理前後で キニーネ塩酸塩刺激した場合の神経電位の変化量を示す 図である。

【図4】実施例3において、本発明抽出剤の処理前後で L-ロイシン刺激した場合の神経電位の変化量を示す図 である。

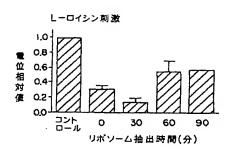
【図5】実施例4において、舌をイオン交換水で洗浄し て得た蛋白質の分析結果を示す図である。

【図6】実施例4において、本発明抽出剤により、抽出 された蛋白質の分析結果を示す図である。

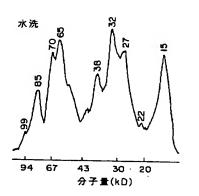
【図7】実施例5において、本発明抽出剤による味覚の 選択的マスキングの結果を示す図である。



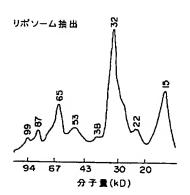
【図4】



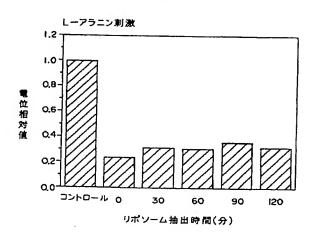
【図5】



【図6】



【図7】



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